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Ochlerotatus japonicus japonicus – a newly established neozoan in Germany and a revised list of the German mosquito fauna

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Abstract

The German Mosquito Control Association (KABS) has been conducting mosquito surveillance as a component of a comprehensive mosquito control programme since 1991. In this study, the results of the first 10 years of this century are presented. Approximately half a million mosquitoes were collected with CO_2-baited adult traps, ovitraps and larval collections mainly in Southwest Germany and were identified to species. A total of 36 autochthonous species and two invasive alien species, namely *Ochlerotatus j. japonicus* [*Hulecoeteomyia japonica* sensu Reinert *et al.,* 2006] and *Aedes albopictus* [*Stegomyia albopicta* sensu Reinert *et al*., 2004] were detected in Southwest Germany. Whereas *Oc. j. japonicus* is already widespread over an area of ≥ 10,000 km^2 in Southwestern Germany and is an established species, *Ae. albopictus* was found only in the egg stage in one ovitrap at a rest-area along a highway from Italy (via Switzerland) where *Ae. albopictus* is abundant. The latter was probably introduced into Germany by vehicles originating from Italy, whereas the introduction of *Oc. j. japonicus* is most probably related to the importation of ornamental plants from Southern Asia (China). *Aedes vexans* [*Aedimorphus* vexans sensu Reinert *et al.,* 2009] is by far the most abundant species in the floodplains of the Rhine Valley with an overall percentage of >68%, followed by *Oc. sticticus* (10.3%) and *Ae. rossicus/Ae. cinereus/Ae. geminus* (>5%). So far, a total of 49 species have been identified in Germany, 48 established species (47 autochthonous and one alien) and one exotic species (*Ae. albopictus*) temporarily imported into Germany.

Key words: *Ochlerotatus j. japonicus*, *Aedes albopictus*, mosquitoes of Germany

Introduction

Mosquitoes disperse passively by wind drift (up to ~15 miles) or by active flight usually limited to <50 km per migration process (Bidlingmayer & Evans, 1987; Becker *et al*., 2010). Present-day human activities enable transportation of mosquitoes from one continent to another within a matter of hours to a few days. Increased transcontinental mobility of humans as well as the international trade, facilitate the dispersal and in some cases, the establishment of exotic mosquito species into other countries with favorable climatic conditions.

Within the Culicidae, 3 species are notable for their dispersal potential and also for their significance as vectors of human diseases: *Aedes aegypti* (Linnaeus) [*Stegomyia aegypti* sensu Reinert *et al*., 2004], *Ae. albopictus* (Skuse) and *Ochlerotatus japonicus japonicus* (Theobald). Their desiccation-resistant eggs and an ability to exploit a wide range of natural and artificial breeding places, enable permanent establishment of viable populations (Hawley, 1988; Moore & Mitchell, 1997).

All 3 species are characterized by their high vector competence for arboviruses. *Aedes aegypti* and *Ae. albopictus* are the primary and secondary vectors of dengue fever (DF) and dengue hemorrhagic fever (DHF) which affects more than 40% of the human population worldwide, especially in mega-cities of the tropics (Halstead, 1980, 1982, 1992; Becker *et al.*, 1991; Gratz, 1999). *Aedes albopictus* is the most important vector for the Chikungunya virus (Reiter *et al*., 2006). Recently, *Ae. albopictus* was involved in the transmission of Chikungunya virus to humans in Italy in 2007 and most probably also in the first confirmed autochthonous dengue cases in France and Croatia in 2010 (Reiter *et al.,* 2006; Beltrame *et al*., 2007; Becker *et al*., 2010; Schmidt-Chanasit *et al*., 2010; Health Protection Report, 2010).

The "African tiger mosquito" /"Yellow fever mosquito", *Ae. aegypti*, has spread across almost all tropical and subtropical countries, during the past four centuries. Populations have increased especially in areas where household water storage in containers is common and where waste disposal services are inadequate. Recently, *Ae. aegypti* was found associated with the Nearctic *Ochlerotatus atropalpus* (Coquillett) [*Georgecraigius atropalpus* sensu Reinert *et al.,* 2006] in used tyres, and *Ae. albopictus* was detected in the Netherlands (Scholte *et al.*, 2010). This is the first record of *Ae. aegypti* in northern Europe for decades (Hansford *et al*., 2010).

The "Asian tiger mosquito", *Ae. albopictus*, originating from Southeast Asia, has undergone a noteworthy expansion of its range in the last few decades (Hawley, 1988). With the increase in international trade in used tyres, this species has spread across very large distances and between continents (Reiter, 1998). In Europe, it was first reported in Albania in 1979 (Adhami & Reiter, 1998) and later in Italy in 1990, where it was introduced through the import of used tyres from the USA into the port town of Genoa (Sabatini *et al*., 1990; Dalla Pozza & Majori, 1992). In the next few years, the species rapidly dispersed to other regions of Italy (Romi, 1994), and now it has been reported from France (Schaffner *et al*., 2000), Serbia and Montenegro (Petric *et al*., 2001), Belgium (Schaffner *et al*., 2004), Switzerland (Flacio *et al*., 2004), Greece (Samanidou *et al*., 2005), Croatia (Klobucar *et al*., 2006), Spain (Aranda *et al*., 2006) and the Netherlands (Scholte *et al*., 2007).

Aedes j. japonicus is an Asian species found in Japan, Korea, South China, Taiwan and the Russian Federation. In 1998, it occurred for the first time in the USA (New Jersey and New York) and is now distributed in at least 22 other states (Saenz *et al*., 2006). *Ochlerotatus j. japonicus* is a competent vector of several arboviruses, including West Nile (WN) virus and Japanese encephalitis (JE) virus and is considered a significant public health risk (Sardelis & Turell, 2001, Sardelis *et al*. 2002a, 2002b, 2003).

The primary dispersal mode of these three invasive mosquitoes by human activity has been through transport of desiccation-resistant eggs in cargo that previously contained stagnant water. The most important type of cargo is used tyres that have been stored outdoors (Knudsen, 1995). Businesses processing or trading used tyres should be given high priority for monitoring of exotic fauna and flora. Another source of introduction is by ornamental plants, e.g. "Lucky Bamboo" (*Dracaena* spp.) from Southeast Asia. For instance, "Lucky

Bamboo" which is transported in containers with standing water, making it an "ideal insectary in transit", was the primary reason for the introduction of *Ae. albopictus* from Southeast Asia to California (Madon *et al*., 2004). Similarly, multiple introductions of the Asian tiger mosquito to the Netherlands in commercial horticultural greenhouses were traced to intensive trade of this plant (Scholte *et al*., 2007, 2010).

Due to high humidity and cool air temperature, the refrigerated transoceanic containers offer ideal conditions suitable for the transport of living insects (Reiter & Darsie, 1984). Therefore, harbours/ ports receiving transoceanic containers, and inland air or road terminals receiving containers from infested countries should be routinely monitored.

Rest areas and parking lots along highways originating in areas infested with exotic species can also serve as sites of introduction (Flacio *et al*., 2004, 2006; Pluskota *et al*., 2008).

Materials and Methods

Routine monitoring of the autochthonous species

Since 1991, the species composition and density of mosquitoes have been monitored with CO_2-baited CDC mosquito traps twice a month between late April and early October at 40 localities along the 280 km of the Upper Rhine Valley between Mainz (State of Rheinland-Pfalz) and Freiburg (State of Baden-Württemberg) in Germany. The traps were set in the late afternoon and removed the next morning in areas with comparable geomorphological and ecological features. The trapped mosquitoes were placed into a container with dry ice. The dead mosquitoes were transferred into glass vials with cotton and kept in the refrigerator until the species composition was determined in the laboratory. The routine trap data yield valuable information about the phenology and population size of the adult mosquitoes. Furthermore, the comparison of the catches in controlled and uncontrolled areas allows an estimation of the reduction of the mosquito population by control operations. In this paper, the results from the years 2000-2009 are evaluated.

In the floodplains of the Rhine River, 100 temporary and semi-permanent breeding sites were monitored monthly from March-October for the developmental stages of mosquitoes. The immature stages were collected in plankton nets and/or standard dippers (WHO), transported to the laboratory in pond water in 1-liter glass containers and reared in "mosquito breeders" to the adult stage. These adults were identified using the keys of Aitken (1954), Mohrig (1969), Becker *et al. (*2003) and Becker *et al*. (2010). Additionally, exuviae of the 4th instar larvae were identified.

Species of the Culex Pipiens Complex (*Culex pipiens* biotype *pipiens* (Linnaeus), *Cx. pipiens* biotype *molestus* (Forskal) and *Cx. torrentium* (Martini), the Anopheles Maculipennis Complex (*Anopheles messeae* (Falleroni)*, An. atroparvus* (Van Thiel) and *An. maculipennis s.s*. (Meigen)) as well as the morphologically similar *Ae. cinereus* (Meigen), *Ae. geminus* (Peus) and *Ae. rossicus* (D.G.M.) were assessed quantitatively, not qualitatively. The eggs of single females of the Anopheles Maculipennis Complex were examined to determine their colour pattern (White, 1978). *Culex pipiens* was assayed for autogeny in large cages to permit estimates of the relative abundance of the anautogenous strongly ornthophilic nominate biotype, and the autogenous *molestus* biotype, which exhibits impartial host preferences. The males of *Cx. torrentium,Cx. pipiens, Ae. rossicus*, *Ae. cinereus* and *Ae. geminus* were identified to species on diagnostic hypopygial structures.

Monitoring of exotic species

a) *Aedes albopictus*

During 2005, the monitoring programme for *Ae. albopictus* was initiated by employing a total of 100 ovitraps at 47 trap sites in the Upper Rhine Valley at resting areas along Highway A5 from Weil am Rhein in the south (close to the Swiss border) to Heidelberg in the north. In addition, some trading companies and container terminals were inspected in the Upper Rhine Valley. From the beginning of May to the end of September of each year, potential sites were monitored every 14 days. The number of ovitraps used per location, varied between 1-6, depending on suitable habitat structures and the probability of *Ae. albopictus* being introduced to the site.

In addition, in the areas surrounding the traps, shrubs and trees were checked for mosquitoes using the human bait method. Eggs were preliminarily identified microscopically and were kept in the laboratory at 25°C and 80% humidity for one week to ensure complete embryonic development. Deoxygenated tap water, enriched with a small amount of brewer's yeast, was used to stimulate hatching. Following hatching, larvae were reared to the adult stage. The exuviae and adults were determined using the identification keys of Becker *et al.* (2003, 2010).

b) *Ochlerotatus japonicus japonicus*

Since the discovery of *Oc. j. japonicus* in northern Switzerland (Schaffner *et al.*, 2009), a monitoring programme was started to evaluate the actual distribution and abundance of this species in Southern Germany. For the first time in September 2009, flower vases in cemeteries, used tyres and water containers in 86 villages close to the Swiss border, were examined for the occurrence of *Oc. j. japonicus* populations. Three teams of two people each checked the breeding sites for the occurrence of developing stages of *Oc. j. japonicus* and associated mosquitoes such as *Cx. pipiens s.l./Cx. torrentium*. The number of positive breeding sites, and at some cemeteries the species composition of each breeding site for *Cx. pipiens s.l./Cx. torrentium* and *Oc. j. japonicus*, were recorded. Immature stages were transported to the laboratory, reared to the adult stage for precise species determination using the keys of Yamaguti & LaCasse (1950), Gutsevitch *et al*. (1971), Tanaka *et al*. (1979) and Becker *et al*. (2003, 2010). The monitoring programme was expanded in 2010. A total of 155 villages in Germany (including the villages examined in 2009), as well as the surrounding areas towards Lake Constance and Black Forest between Weil am Rhein, Radolfzell, Freiburg and Donaueschingen in the north, were checked to assess the extent of dispersal of this invasive species.

The container index for *Oc. j. japonicus* and *Cx. pipiens s.l./Cx. torrentium* was determined in 25 cemeteries which contained at least 20 breeding sites.

Results

Routine Monitoring:

A total of 408,716 female mosquitoes belonging to 25 species or species complexes (Table 1) were collected in the floodplains with CO_2 traps. *Aedes vexans* (Meigen) was the most abundant species (68.5%), followed by *Oc. sticticus* (Meigen) (10.3%,), *Ae. rossicus* (5.4%), *Ae. cinereus/Ae. geminus* (>5%). *Cx. pipiens s.l./Cx. torrentium*, *Cx. modestus* (Ficalbi) and

Oc. annulipes (Meigen) amounted to <2% of all mosquitoes trapped in the floodplains and numbers of all other species amounted to <1 %.

Species abundance was assessed within the larval populations from 100 breeding sites**.** A total of ~10,000 larval identifications revealed the presence of 25 species. *Aedes vexans* was predominant, with ~70% of all collected larvae and often >90% in some extreme temporary breeding sites when flooding occurred between May-September. Larvae of *Oc. sticticus*, *Oc. cantans* (Meigen), *Oc. annulipes*, *Ae. rossicus*, *Ae. cinereus*, *Cx. pipiens s.l.*, *Cx. torrentium*, *Cx. modestus*, *Cs. annulata* (Schrank), *An. maculipennis s.l.* and *An. claviger s.s*. (Meigen) were relatively abundant in the floodplains. All other species, *Ae. geminus*, *Oc. nigrinus* (Eckstein), *Oc. caspius* (Pallas), *Oc. detritus* (Haliday), *Oc. leucomelas* (Meigen), *Oc. flavescens* (Müller), *Oc. cataphylla* (Dyar), *Oc. rusticus* (Rossi), *Oc. communis* (De Geer) and *Cs. morsitans* (Theobald) were less abundant. The ornithophilic *Uranotaenia unguiculata* was found in the northern part of the Upper Rhine valley, in eutrophic breeding sites such as shallow oxbows or ditches polluted with organic material. *Culex territans* (Walker) [*Culex europaeus* of Da Cunha Ramos *et al*., 2003)] which prefers blood meals from amphibians, occurred only in larger numbers in areas with high densities of *Rana esculenta s.l.* and *Hyla arborea*.

Aedes albopictus: In the course of the ovitrap monitoring program, a total of four species of culicids were collected. Most of them belonged to the indigenous dendrolimnobiotic species *Aedes geniculatus* (Olivier) [*Dahliana geniculata* sensu Reinert *et al.,* 2006] and *An. plumbeus* (Stephens), as well as in some cases, *Culex pipiens s.l./Cx. torrentium*. However, in September 2007, five eggs from the non-indigenous *Ae. albopictus* were found on the ovipositional substrate in one of the ovitraps at a parking lot along the southern part of the German Highway A5 (Latitude 47°42'22"N, Longitude 7°31'28"E). Only two of the five eggs hatched, both larvae developed into adults and were conclusively identified as *Ae. albopictus*. No eggs were found in the second ovitrap at the same site, nor were adults found up to 200m around the traps, using the human bait method.

Ochlerotatus j. japonicus: In 2009, of the 86 villages monitored for *Oc. j. japonicus,* immature stages were found in cemeteries of 25 villages (32.6%), especially in the area east of Waldshut-Tiengen (Fig 1). In 2010, of the 155 villages monitored, cemeteries in 50 villages (32.3%) were infested with immature stages of *Oc. j. japonicus*. Villages towards Weil am Rhein which were not infested with *Oc. j. japonicus* in 2009, were positive for immature stages in 2010. Even in the mountainous area of the Black Forest (Bernau) at an elevation of ~1,200m, immature stages of *Oc. j. japonicus* were recorded.

For the assessment of the container index, 755 containers (mainly flower vases at the cemeteries) were investigated. A total of 18.7% were positive, 8.2% were inhabited by *Oc. j. japonicus* and 10.5% by *Cx. pipiens s.l./Cx. torrentium*. Seven of 755 (0.9%) breeding sites contained mixed populations. Larvae of *Oc. j. japonicus* were more abundant than *Cx. pipiens s.l./Cx. torrentium* at 12 cemeteries.

Discussion

The remarkable result of this study is the record of *Oc. j. japonicus* as an established and already widespread species at least in Southwest Germany. The results of this study show an area of ~2,200km^2 infested with *Oc. j. japonicus*. Schaffner *et al*. (2009) found an area of ~1,400km^2 infested by this species in northern Switzerland. In Germany (as in Switzerland), *Oc. j. japonicus*, though mainly found in flower vases at cemeteries, is also present in used tyres and rainwater storage containers. In future studies, it is intended to gain additional information about the occurrence of this species in tree holes and other potential breeding sites.

Since *Oc. j. japonicus* has become established as an exotic invasive species in Germany, the number of mosquito species found in Germany has increased to 49 (47 autochthonous species, *Oc. j. japonicus* as an established invasive species and *Ae. albopictus* as sporadic imported species). In addition to the 36 identified species occurring in the Upper Rhine Valley, the authors found larvae of *Oc. pullatus* (Coquillett) and *Cs. ochroptera* (Peus) in boggy waters (Hinterzartner Moor) in the Black Forest (Becker & Ludwig, 1981). Mohrig (1969) reports the occurrence of the following nine species in Germany which were not found during our investigations (*Oc. cyprius* (Ludlow), *Oc. intrudens* (Dyar), *Oc. refiki* (Medschid), *Oc. riparius* (Dyar and Knab), *An. labranchiae* (Falleroni), *Cx. hortensis* (Ficalbi), *Cx. martinii* (Medschid), *Cs. fumipennis* (Stephens) , *Cs. glaphyroptera* (Schiner)).

Ochlerotstus j. japonicus is the second German record of a newly established species (after *Ur. unguiculata*) within the last 15 years (Becker & Kaiser, 1995). The regular occurrence of the latter was also confirmed in this study by additional records of developing stages in two different states in southwest Germany (Rheinland-Pfalz and Hessen). The number of recorded species in southwest Germany increased to 38, with 37 autochthonous, one established neozoan (*Oc. j. japonicus*) and one occasionally occurring species *Ae. albopictus* (Table 2). Eckstein (1920) reported 18 species in the area of Mannheim, Scherpner (1920) 26 species in the area of Frankfurt and Becker and Kaiser (1995) 34 species in the Upper Rhine Valley.

Ochlerotatus j. japonicus (like *Ae. albopictus*) is a successful invasive species. As in the USA, where it is currently present in >22 States (Saenz *et al,*. 2006), this species may perhaps quickly establish stable populations over the next few years in southern Germany and Switzerland (Schaffner *et al*., 2009). Obviously, the successful colonization of new areas in Germany and Switzerland is favoured by the moderate climate, which is similar to that of its native range in Asia. In North America, it is currently broadening its range and presently occurs in >22 US states (Saenz *et al*., 2006). It was intercepted in New Zealand in a shipment of used tyres, which was probably also the pathway of introduction into the USA. The pathway of introduction to Germany is not yet clear. One hypothesis is that this species was introduced via used tyres or by airfreight through Zürich. However, it seems that this species is most abundant in flower vases in cemeteries indicating that used tyres may not be the only reason for the widespread occurrence. A further possibility is that *Oc. j. japonicus* was, and maybe still is, introduced together with ornamental plants (e.g. the box tree *Buxus* spp.) in transoceanic containers originating from Asia. *Buxus* spp. are common plants in cemeteries and are frequently imported from eastern Asia. A strong indication, that *Oc. j. japonicus* is imported together with plants from eastern Asia, is the fact that another neobiota, namely the box tree pyralid, *Glyphodes perspectalis* (Walker 1859) (Lepidoptera: Crambidae) occurs in the vicinity of Basel, Lörrach and Rheinfelden and also in Aargau and other parts of northern Switzerland. This invasive alien moth has damaged box trees since about 2007 in the area where it is most abundant (Billen, 2007; Van der Straten & Muus, 2010). Both insects, the moth and the mosquito, occur in China, Japan and Korea. It can be surmised that both species were introduced at the same time to parts of Europe via the same trade route with ornamental plants such as Box trees. After the moth was first recorded in Germany 2007, it has now been observed in six European countries and continues to spread. A pathway for the introduction of the neobiota to southern Germany could be the harbours along the Rhine River close to Basel and Weil am Rhein.

The first record of *Ae. albopictus* eggs in the southern part of the Upper Rhine Valley, is proof that this species is being introduced by vehicles, most probably by tourists from Italy (via Switzerland) to Germany on Highway A5, especially during vacation periods, a further sign for the globalization of mosquitoes (Flacio *et al*., 2006; Pluskota *et al*., 2008; Becker, 2009).

The fact that only five eggs were found in only one trap and that no adults were collected, implies that the eggs were laid by a single transported female that was already inseminated. *Aedes albopictus* females lay an average of 62.5 eggs (Gubler, 1970) and tend to deposit these in multiple ovipositional sites (Hawley, 1988). Therefore, it is likely that more eggs from this female could have been found in natural or man-made containers at this site. However, it is very unlikely that a stable population can be established with such a small original population (Hanski, 1999). Perhaps, additional mosquitoes introduced in the future could increase the risk for the establishment of an exotic mosquito species in Germany. The changing climatic conditions reflected by increasing temperatures will further support the spread of species such as *Ae. albopictus* in Central Europe (Becker, 2009). The occurrence of *Oc. j. japonicus* in the mountainous area of the Black Forest up to an elevation of >1,000m indicates that this species finds climatic conditions in Germany similar to its countries of origin where it also occur at higher elevations (Yamaguti & LaCasse, 1950).

Control of an invasive mosquito species in Germany should incorporate four major elements:

a) Evaluation of the pathways of the introduction of exotic invasive species. The goal is to prevent further invasions of neobiota by governmental regulating agencies and with close cooperation with trading companies.
b) Increasing mosquito monitoring activities to assess the actual or suspected infestation areas of exotic species by routine site inspections and trapping.
c) Information to the public will be disseminated by regular press releases. Thorough information about the invasive mosquito species will be provided on the internet.
d) Development of control strategies to reduce or perhaps eliminate the invasive exotic mosquito species. All tools at our disposal will be employed as sson as an introduction is detected, including larviciding, adulticiding and an aggressive public education program encouraging community participation to reduce potential breeding sites.

Close cooperation by all European countries is instrumental in combatting exotic, invasive mosquito species and imported parasites/pathogens which pose an increasing threat to the human population. The re-emergence of dengue cases in the Mediterranean area in 2010 and the outbreak of Chikungunya fever in Italy 2007 as well as the occurrence of new arboviruses in Germany in 2010 are significant warning signs, that serious efforts to solving these problems are necessary (Jöst *et al*., 2010; Schmidt-Chanasit *et al*., 2010; Talbalaghi *et al*., 2010).

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Table 1: No of mosquitoes collected with carbon dioxid traps in the Upper Rhine Valley from 2000 - 2009

Species	**2000**	**(%)**	**2001**	**(%)**	**2002**	**(%)**	**2003**	**(%)**	**2004**	**(%)**	**2005**	**(%)**	**2006**	**(%)**	**2007**	**(%)**	**2008**	**(%)**	**2009**	**(%)**	**total no.**	**total %**
Ae. vexans	13,935	64.8	8,505	29.6	39,225	81.3	272	17.5	51,624	85.8	43,894	77.5	19,116	39.0	39,256	71.1	31,767	70.9	32,399	75.1	279,993	68.5
Oc. sticticus	621	2.9	3,885	13.5	2,005	4.2	22	1.4	5,291	8.8	5,850	10.3	7,317	14.9	8,327	15.1	3,678	8.2	5,246	12.2	42,242	10.3
Ae. rossicus	23	0.1	773	2.7	919	1.9	-	-	627	1.0	2,881	5.1	11,280	23.0	2,512	4.5	2,399	5.4	952	2.2	22,366	5.4
Ae.cinereus/geminus	960	4.5	3,414	11.9	1,754	3.6	50	3.2	900	1.5	2,229	3.9	6,765	13.8	2,240	4.4	1,684	3.8	992	2.3	20,988	5.1
Cx. pipiens/torrentium	1,408	6.5	1,021	3.6	992	2.1	259	16.7	462	0.8	339	0.6	1,003	2.0	780	1.4	629	1.4	1,202	2.8	8,095	1.9
Cx. modestus	1,623	7.5	3,320	11.5	444	0.9	600	38.6	455	0.8	134	0.2	567	1.2	353	0.6	125	0.3	174	0.4	7,795	1.9
Oc. annulipes	327	1.5	2,560	8.9	524	1.1	32	1.1	67	0.1	386	0.7	1,155	2.4	189	0.3	1,504	3.4	698	1.6	7,442	1.8
Cq. richiardii	465	2.2	1,137	4.0	1,059	2.2	80	5.1	82	0.1	64	0.1	146	0.3	214	0.4	331	0.7	-	-	3,578	0.8
Oc. cantans	549	2.6	800	2.8	382	0.8	26	0.8	85	0.1	125	0.2	647	1.3	120	0.2	603	1.3	211	0.5	3,548	0.8
An. maculipennis s.l.	140	0.7	669	2.3	206	0.4	120	7.7	329	0.5	278	0.5	366	0.7	211	0.4	681	1.5	203	0.5	3,203	0.7
An. plumbeus	239	1.1	421	1.5	273	0.6	28	1.8	55	0.1	100	0.2	117	0.2	200	0.4	415	0.9	277	0.6	2,125	0.5
Cs. annulata	288	1.3	470	1.6	220	0.5	39	2.5	128	0.2	152	0.3	157	0.3	311	0.6	216	0.6	107	0.2	2,088	0.5
An. claviger	646	3.0	380	1.3	45	0.1	11	0.7	56	0.1	61	0.1	32	0.1	236	0.4	46	0.1	48	0.1	1,561	0.3
Oc. punctor	113	0.5	844	2.9	71	0.1	4	0.3	2	<0.1	48	0.1	59	0.1	13	<0.1	91	0.2	24	0.1	1,269	0.3
Oc. communis	87	0.4	236	0.8	54	0.1	3	0.2	-	-	32	0.1	83	0.2	28	0.1	127	0.3	118	0.3	768	0.1
Oc. rusticus	49	0.2	245	0.9	53	0.1	4	0.3	1	<0.1	22	<0.1	150	0.3	12	<0.1	187	0.4	1	0.1	724	0.1
Oc. diantaeus	13	0.1	50	0.2	24	<0.1	3	0.2	1	<0.1	9	<0.1	106	0.2	19	<0.1	259	0.6	121	0.3	605	0.1
Cx. morsitans	3	<0.1	4	<0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	<0.1
Oc. geniculatus	10	<0.1	8	<0.1	6	<0.1	1	0.1	1	<0.1	5	<0.1	9	<0.1	5	<0.1	12	<0.1	9	<0.1	66	<0.1
Oc. caspius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	<0.1	1	<0.1	-	-	11	<0.1
Oc. flavescens	3	<0.1	1	<0.1	2	<0.1	1	0.1	-	-	-	-	1	<0.1	-	-	-	-	-	-	8	<0.1
Oc. cataphylla	-	-	-	-	-	-	-	-	-	-	1	<0.1	-	-	-	-	-	-	-	-	1	<0.1
Oc. leucomelas	3	<0.1	2	<0.1	-	-	-	-	-	-	-	-	-	-	-	-	3	<0.1	-	-	8	<0.1
Total	**21,505**		**28,745**		**48,258**		**1,555**		**60,166**		**56,610**		**49,076**		**55,216**		**44,803**		**42,782**		**408,716**	

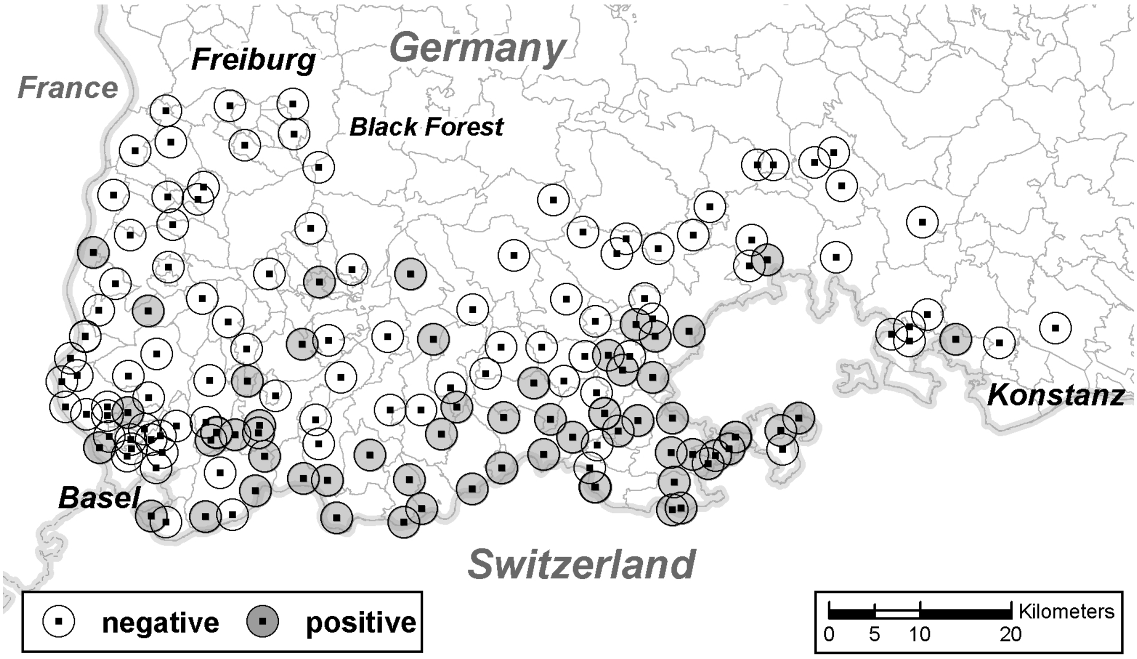

Figure 1: Investigated villages in Southwest Germany with cemeteries infested with *Oc. j. japonicus* in 2009 and 2010.

Author	Eckstein	Scherpner	Becker/Kaiser	Becker *et al.*	Total no. of species
Species	*(1920)*	*(1960)*	*(1995)*	*(2010)*	*(2011)*
Ae. vexans	++++	++	++++	++++	Recorded
Oc. sticticus	+++	+++	+++	+++	Recorded
Ae.cinereus	+++	++++	++	++	Recorded
Ae. geminus	?	?	proven	proven	Recorded
Ae. rossicus	?	?	++	++	Recorded
*Ae. albopictus**	-	-	-	[(+)]	[not established]
Oc. caspius	-	(+)	+	(+)	Recorded
Oc. dorsalis	+	-	-	(+)	Recorded
Oc. detritus	-	-	(+)	(+)	Recorded
Oc. nigrinus	-	-	(+)	(+)	recorded
Oc. leucomelas	-	(+)	+	+	recorded
Oc. flavescens	-	-	(+)	(+)	recorded
Oc. intrudens	-	-	-	-	recorded
Oc. annulipes	(+)	+	+	++	recorded
Oc. cantans	++++	+++	++	++	recorded
Oc. cataphylla	-	+	(+)	(+)	recorded
Oc. excrucians	-	(+)	(+)	(+)	recorded
Oc. rusticus	+	+	+	++	recorded
Oc. refiki	-	-	-	-	recorded
Oc. communis	++	+++	+	+	recorded
Oc. cyprius	-	-	-	-	recorded
Oc. punctor	-	(+)	+	+	recorded
Oc. riparius	-	-	-	-	recorded
Oc. diantaeus	-	-	(+)	(+)	recorded
Oc. pullatus	-	-	-	(+)	recorded
Oc. geniculatus	+	+	(+)	(+)	recorded
Oc. japonicus	-	-	-	+	recorded
Cx. pipiens s.l.	++++	++++	++++	++++	recorded
Cx. p. biotype *pipiens*	?	[proven]	[proven]	[proven]	[recorded]
Cx. p. biotype *molestus*	?	[proven]	[proven]	[proven]	[recorded]
Cx. torrentium	?	+++	proven	proven	recorded
Cx. hortensis	-	-	-	-	recorded
Cx. martinii	-	-	-	-	recorded
Cx. modestus	-	-	++	++	recorded
Cx. territans	++	(+)	(+)	++	recorded
Cs. annulata	++	++	++	++	recorded
Cs. morsitans	++	+	+	+	recorded
Cs. subochrea	-	(+)	(+)	(+)	recorded
Cs. ochroptera	-	-	-	+	recorded
Cs. fumipennis	-	-	-	-	recorded

Cs. alascaensis	-	-	(+)	(+)	recorded
Cs. glaphyroptera	-	-	-	-	recorded
An. maculipennis s.l.	++++	[+++]	[++]	[+++]	[recorded]
An. messeae	?	+++	proven	proven	recorded
An. atroparvus	?	++	proven	proven	recorded
An. maculipennis s.s.	?	+	proven	proven	recorded
An. labranchiae	-	-	-	-	recorded
An. claviger	+++	++	+	++	recorded
An. algeriensis	-	(+)	-	-	recorded
An. plumbeus	+	+	+	++	recorded
Cq. richiardii	++	+	+	+	recorded
Ur. unguiculata	-	-	(+)	(+)	recorded
Number of species	**17**	**26**	**34**	**38**	**48**

Table 2: Recorded mosquito species from 1920 to 2010 in Germany (occurrence: ++++ = massive; +++ = abundant; ++ = frequent; + = regularly; (+) = rare; * species is found only sporadic; ? = uncertain; [ ] = not counted in the species lists.